REMARKS

This amendment adds, changes, and/or deletes claims in this application. Specifically, claims 28 and 34-38 are canceled, claims 29-33 are amended, and claims 39-60 are added. A detailed listing is set forth above of all pending claims, with appropriate defined status identifiers. Upon entry of these amendments, claims 29-33 and 39-60 will be pending. Applicant respectfully requests reconsideration of those claims.

Examiner Interview

Applicant thanks Examiner Kallis and Supervisory Examiner Nelson for the courtesies extended to Applicant's representative during the interview conducted on June 7, 2004. Applicant believes that the interview was very helpful in advancing prosecution of the application. In accordance with MPEP § 713.04, the substance of the interview, which also is reflected in the Interview Summary, is discussed here with reference to specific issues, including claim language, plant nonsymbiotic hemoglobins, incorporation by reference, written description, enablement, and prior art.

Claim Amendments

The foregoing amendments revise claims, previously directed to a "method of improving agronomic properties," to recite methodology for "increasing a plant's tolerance to hypoxic conditions," and to recite recombinant techniques, as discussed during the interview. Support for new claim 39 and for related changes to claims 30-33 is found throughout the original specification. For example, page 3 states that the invention provides "a method of increasing tolerance to hypoxic conditions," and canceled claim 34 recited a method that provides "increased tolerance to hypoxic conditions." The recombinant aspect of these claims is supported, for example, at pages 2-3 of the specification as filed, which state that the invention provides recombinant expression systems for expressing non-symbiotic hemoglobin (ns-Hb) and cells and organisms transformed therewith. *See also* page 23. Support for the recitation of plant ns-HB and for transformed plants is found throughout the specification. *See, e.g.*, pages 1-2 (discussing plant ns-Hbs generally) and page 23 (discussing the transformation of plants generally). Support for the comparative language is

found throughout the specification and in the examples, which discuss differences between transformed and non-transformed plants under hypoxic conditions.

New claims 40-54 recite specific embodiments related to the method of claim 39. Support for these claims in the specification as filed is outlined below:

New claim 40 relates to different hypoxic conditions, and is supported, for example, by the teachings at page 9 of the specification as filed.

New claim 41 recites the property of increased cellular metabolism, and is supported, for example, by the teachings at page 9 of the specification as filed.

New claims 42-50 are supported, for example, at page 2 (control sequence), page 3 (strong constitutive promoter), page 23 (host-specific promoter), page 2 (rice ns-Hb and *Arabidopsis* ns-Hb), page 20 (maize ns-Hb), page 8 (transformation of maize, maize ubiquitin promoter, selectable marker).

New claim 51 recites a plant made in accordance with the method of claim 39, and is supported throughout the specification, for example at page 23.

New claims 52 and 53 recite transformed plants that express plant ns-Hb at elevated levels, and are supported, for example, at page 11 of the specification.

New claim 54 recites a method of determining if a seed is germinating (previously recited in canceled claim 37) and includes "correlating," as suggested during the interview. This claim is supported, for example, at pages 5 and 8 and by Example X at pages 14-16.

New claims 55-60, which depend from claim 54, are directed to specific embodiments of the method of claim 54, and are supported, for example, by Example X at pages 14-16.

Applicant believes that the instant claims are in condition for allowance, conforming as they do to the suggestions made by Supervisory Examiner Nelson and Examiner Kallis during the interview.

Non-Symbiotic Hemoglobins

During the interview, Supervisory Examiner Nelson questioned whether "non-symbiotic hemoglobins" are distinguished from other hemoglobins. Applicant responds that ns-Hbs are known in the art as a distinct class of plant hemoglobins.

As the specification states on page 1, plant hemoglobins have been classified into symbiotic and non-symbiotic hemoglobins. While symbiotic hemoglobins are found in plants capable of participating in microbial symbiosis (where they play a role in regulating oxygen supply to nitrogen fixing bacteria), ns-Hbs are ubiquitous in plants. The publications provided in Appendix A to this response evidence the recognition in the art, by the time the present application was filed, that ns-Hbs constituted a distinct class of plant hemoglobins. The publications in Appendix A include: Trevaskis *et al.*, *Proc. Nat'l Acad. Sci. USA* 94: 12230-34 (1997) (pg. 12230, col. 1, 1st ¶ after the Abstract); Andersson *et al.*, *Proc. Nat'l Acad. Sci. USA* 93: 5682-87 (1996) (pg. 5682, col. 1, 3rd ¶ after the Abstract); and Arrendondo-Peter, *Plant Physiol.* 115: 1259-66 (1997) (pg. 1259, paragraph bridging cols. 1-2).[1] Thus, those skilled in the art readily will recognize the metes and bounds of plant "nonsymbiotic hemeglobins," and will know them to be separate from other plant hemeglobins, *i.e.*, symbiotic hemeglobins such as leghemoglobins.

Incorporation By Reference

Page 2 of the Office Action objects to the specification, contending improper incorporation by reference of "essential material" from the 1997 Duff paper. As indicated at the interview, Applicant has reviewed the specification and the Duff paper and does not believe that the latter is essential to the description or enablement of the invention. In particular, Duff describes the cloning, expression in *E. coli*, purification, and study of barley ns-Hb. While similar methods can be used to carry out certain aspects of the claimed invention, the instant application need not include the specific disclosures of Duff in order to satisfy § 112. Indeed, other portions of the specification provide ample teachings to enable

[1] Each of these references has been made of record in this application by the submission of Information Disclosure Statements.

those skilled in the art to practice the claimed methods. Accordingly, applicant has amended the specification to delete the incorporation by reference. Applicant also notes that the Duff paper was published before the filing date of the application and, hence, effectively was part of the conventional knowledge that the skilled person would bring to a reading of the application.

Written Description

Pages 3-4 of the Office Action reject the claims for an alleged lack of written description because the specification does not expressly set forth any ns-Hb nucleotide sequences. Applicant respectfully traverses this rejection.

As discussed during the interview, a key aspect of the present invention lies not in the isolation or sequencing of ns-Hb sequences, but rather in transforming a plant with an expression system that comprises a nucleic acid molecule encoding a plant nonsymbiotic hemoglobin (claim 39 and dependents) and in determining, from ns-Hb expression levels, whether a seed is germinating (claim 54 and its dependents). Although these methods use ns-Hb nucleic acid sequences or involve detecting their expression, the invention does not provide novel ns-Hb sequences *per se*.

When the present application was filed, a number of ns-Hb sequences were known. As taught at page 1 of the specification, Taylor *et al.*, *Plant Mol Biol* 24: 853-62 (1994), discloses a **barley** ns-Hb nucleotide sequence. Arrendondo-Peter 1997, *supra*, discussed at page 2 of the specification, discloses a **rice** ns-Hb sequence. Trevaskis 1997, *supra*, also discussed at page 2 of the specification, describes the expression an ***Arabidopsis*** ns-Hb sequences, and provides a GenBank deposit number for it. Additionally, Andersson 1996, *supra*, describes a **soybean** ns-Hb sequences and provides a GenBank deposit number for it, and Jacobsen-Lyon *et al.*, *Plant Cell* 7: 213-23 (1995), describes an ns-Hb from ***Casuarina glauca***. Copies of these publications appear in Appendix A, for the Examiner's convenience.[2] Apart from disclosing additional specific ns-Hb sequences, these publications

[2] Each of these references has been made of record in this application by the submission of Information Disclosure Statements.

are believed to be cumulative of those cited in the specification and of record in the application.

Because nucleic acid molecules encoding plant ns-Hbs were known when the application was filed, the Federal Circuit's *Eli Lilly* decision, which addressed claims reciting novel cDNAs, simply is not relevant. Moreover, because the claimed invention is not directed to novel ns-Hb sequences *per se*, the specification need not describe such sequences with the level of detail required by *Enzo*. The reference in the specification to ns-HB sequences, and the citation (for instance, at page 2) of an exemplary number of known ns-Hb sequences, satisfies the written description requirement with respect to the recitation of nucleic acid molecules encoding plant nonsymbiotic hemoglobin. Applicant therefore respectfully urges the Examiner to reconsider and withdraw this rejection.

Enablement Rejection

The Office Action, at pages 4 and 7, rejects the claims for an alleged lack of enablement. Applicant respectfully traverses these rejections for the reasons set forth below.

On pages 5 and 6, the Office Action asserts that a methodology including the breeding of plants to increase ns-Hb levels is not enabled. Without acquiescing to this proposition, and without prejudicing the option of pursuing the original subject matter in a continuing application, Applicant has amended the claims to recite recombinant methods of increasing ns-Hb levels. The rejection regarding the breeding embodiment of the invention therefore is moot.

On pages 6 and 7, the Office Action contends that an undue amount of experimentation would have been required to isolate DNA sequences encoding ns-Hbs from any plant species. As demonstrated above, however, a number of plant ns-Hb sequences already were known and accessible to the artisan when the application was filed. Accordingly, little if any experimentation would have been required to obtain nucleic acid molecules suitable for use in the present invention, and this rejection therefore should be withdrawn.

Applicant also notes that those skilled in the art readily could have used methods routine in the art, such as those described in the references included in Appendix A, to obtain other ns-Hb sequences. Indeed, since the application was filed, ns-Hb sequences have been reported for other plants, including *Cichorium* (Hendriks *et al.*, *Biochim. Biophys. Acta.* 1443: 193-97 (1998), *Lotus japonica* (Uchiumi *et al.*, *Plant Cell Physiol.* 43: 1351-58 (2002)), wheat and potato (Larsen, *Biochim. Biophys. Acta.* 1621: 299-305 (2003)), and *Euryale ferox* (Guldner *et al.*, *J. Evol. Biol.* 17: 48-54 (2004)). Copies of publications are included in Appendix B for the Examiner's convenience. These papers support Applicant's position that no undue amount of experimentation would have been required to practice the invention with respect to obtaining nucleic acid molecules encoding a plant ns-Hb.

Page 7 of the Office Action asserts that the claims are not enabled because the specification has "not taught how to use any hemoglobin in a whole plant." Applicant respectfully disagrees.

The teachings in the specification are sufficient to enable those skilled in the art to transform a whole plant with an expression system comprising a nucleic acid molecule encoding a plant nonsymbiotic hemoglobin. For example, page 23 provides teachings on expression vectors useful in a variety of host organisms, to induce expression of ns-Hb in host organisms such as plants. Moreover, the specification includes a number of examples relating to the successful transformation of maize cells with barley ns-Hb. Although these examples do not demonstrate transformation of whole plants *per se*, the methodology is generally applicable. The Office Action does not include any evidence or reasoning to the contrary.

As discussed during the interview, the enabling quality of the specification is supported by post-filing date publications reporting the transformation of whole plants with nucleic acid molecules encoding ns-Hb, as taught in the specification. For example, Dordas *et al.*, *Plant J.* 35: 763-70 (2003), reports alfalfa plants transformed with barley ns-Hb. Additionally, Seregelyes *et al.*, *Acta Biol. Hung.* 54: 15-25 (2003), discloses tobacco plants transformed to overexpress alfalfa ns-Hb (*see also,* Seregelyes *et al.*, manuscript draft). Copies of these papers are included in Appendix C, for the Examiner's convenience. These papers demonstrate that those skilled in the are were able to transform whole plants with ns-

Hb sequences in accordance with the teachings of the specification without an undue amount of experimentation.

Page 7 of the Office Action also asserts that the claims are not enabled because of concerns that all tissues may not "tolerate increases in the cellular level of hemoglobin without compromising other essential cellular activities." The Office Action does not set forth any basis for this concern. As taught in the specification, ns-Hb appears to function at a metabolic level to maintain a cell's energy status under hypoxic conditions. There is no reason to assume that this function might somehow interfere with essential cellular activities. Indeed, as demonstrated by the examples, transformation with ns-Hb permits such activities to continue under hypoxic conditions, as indicated by continued growth, maintained ATP levels, and oxygen uptake. Moreover, the examples in the specification show that transformed cells appear to function no differently than non-transformed cells under non-hypoxic conditions, such as when grown in an air environment. *See, e.g.,* Examples VI-IX at pages 11-14.

In view of the foregoing, Applicant believes that the instant record demonstrates that those skilled in the art at the time the application was filed could have practiced the claimed invention without an undue amount of experimentation. Applicant therefore respectfully urges the Examiner to reconsider and withdraw the enablement rejections.

Prior Art

Pages 8-10 of the Office Action set forth prior art rejections of the claims in view of Taylor and Andersson (claims 24-34) and Taylor and Hartl (claims 28-38). As discussed during the interview, these references do not teach or suggest the invention of the instant claims. With regard to independent claim 39 and dependent claims 29-33 and 40-53, Applicant notes that the references do not suggest transforming a plant with a nucleic acid molecule encoding a plant ns-Hb. With regard to claims 55-60, Applicant notes that the references do not suggest correlating the level of ns-Hb expression with germination. Accordingly, Applicant respectfully urges withdrawal of these prior art rejections.

CONCLUSION

In view of the foregoing, Applicant believes that the application is in condition for allowance, and an early notice to that effect is earnestly solicited.

Should there be any questions regarding this submission, or should any issue remain, the Examiner is invited to contact the undersigned attorney at the telephone number set forth below.

The Commissioner is hereby authorized to charge any additional fees which may be required regarding this application under 37 C.F.R. §§ 1.16-1.17, or credit any overpayment, to Deposit Account No. 19-0741. Should no proper payment be enclosed herewith, as by a check being in the wrong amount, unsigned, post-dated, otherwise improper or informal or even entirely missing, the Commissioner is authorized to charge the unpaid amount to Deposit Account No. 19-0741. If any extensions of time are needed for timely acceptance of papers submitted herewith, Applicant hereby petitions for such extension under 37 C.F.R. §1.136 and authorizes payment of any such extensions fees to Deposit Account No. 19-0741.

Respectfully submitted,

Date June 15, 2004

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